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In vitro Characterization of Recombinant Human BuChE (Protexia) as a Potential Bioscavenger

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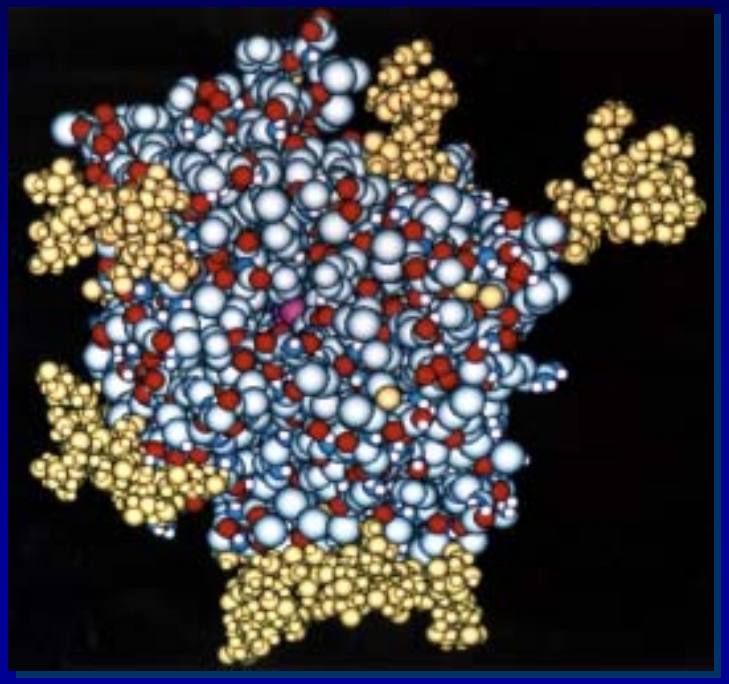
Objectives

- **Demonstrate improved medical protection against nerve agents**
- **Develop a prophylactic that detoxifies nerve agents at a rate sufficient to protect against $5LD_{50}$ exposure**
- **Prophylactic should:**
  - Be non-toxic
  - Produce no adverse side effects
  - Have no adverse effect on performance
  - Be easy to administer
  - Have a long biological half-life



3-D model of BuChE allows for rational drug design and molecular biology approaches to development of biological scavengers.

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Concept

- Provide a pretreatment capable of protecting against up to 5xLD_{50} of nerve agent with no side effects and no need for additional protective clothing or therapy

Potential Bioscavengers

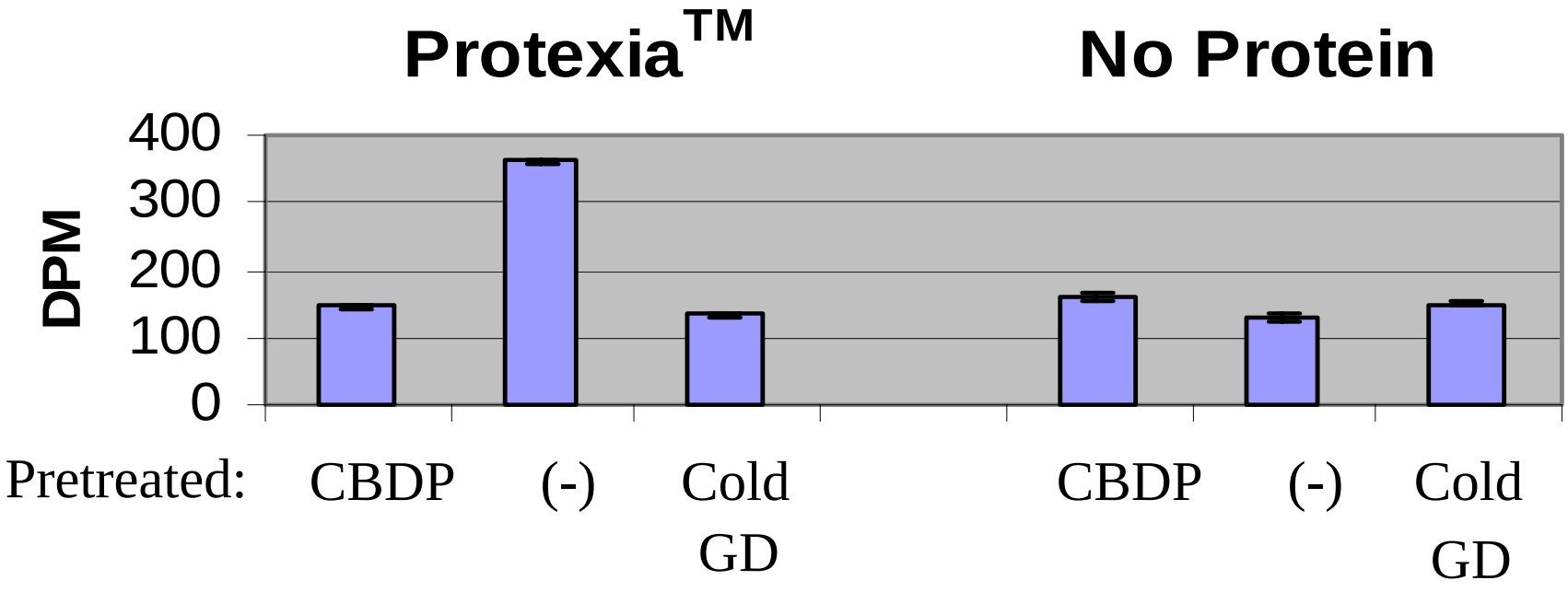
Efforts to date have focused on BuChE of human origin

- Production of Hu BChE From Cohn Fraction IV-4
  - single band on SDS-PAGE
  - ~ 20 g =14 million units of purified BChE
  - >98% pure; specific activity ~700 U/mg

- Material (Protexia™) from milk of transgenic goats is now available
  - Hu-BuChE gene fused to a milk promoter
  - Expression is directed to the milk of transgenic goats
  - Purified material has Specific activity ~595 U/mg

Need to characterize Protexia™ versus plasma derived material

Protexia™ Binds H³-Soman



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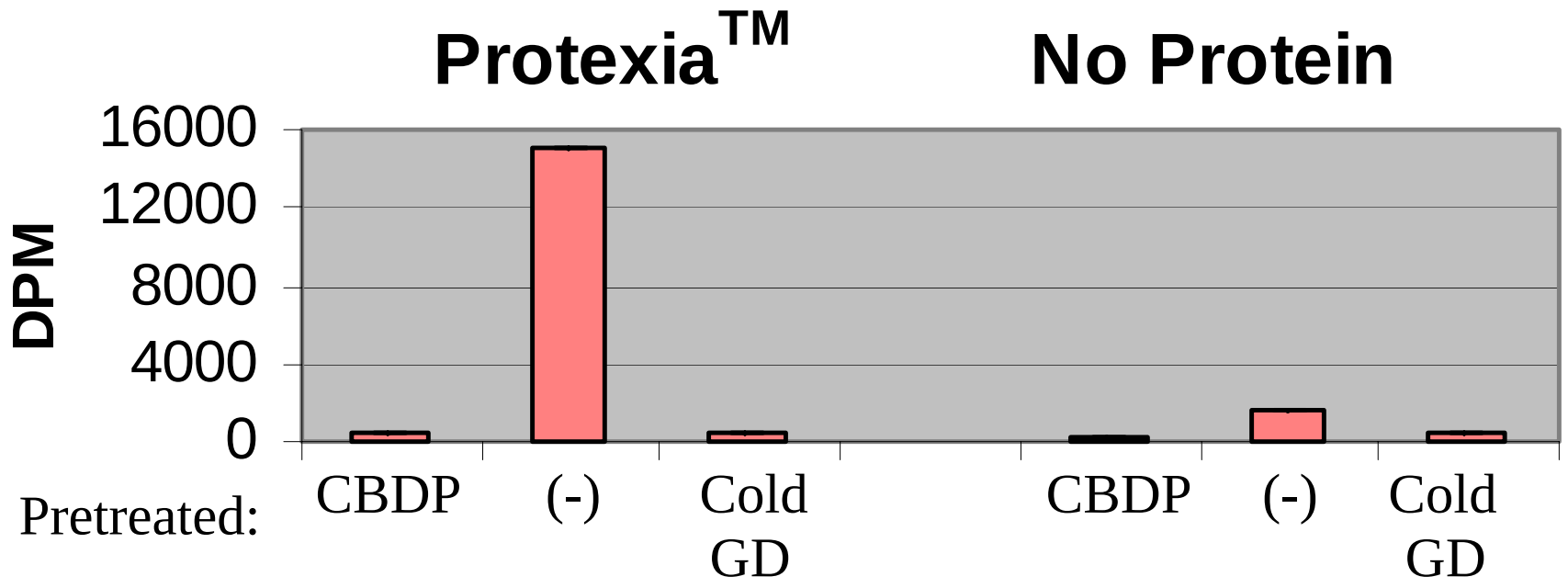
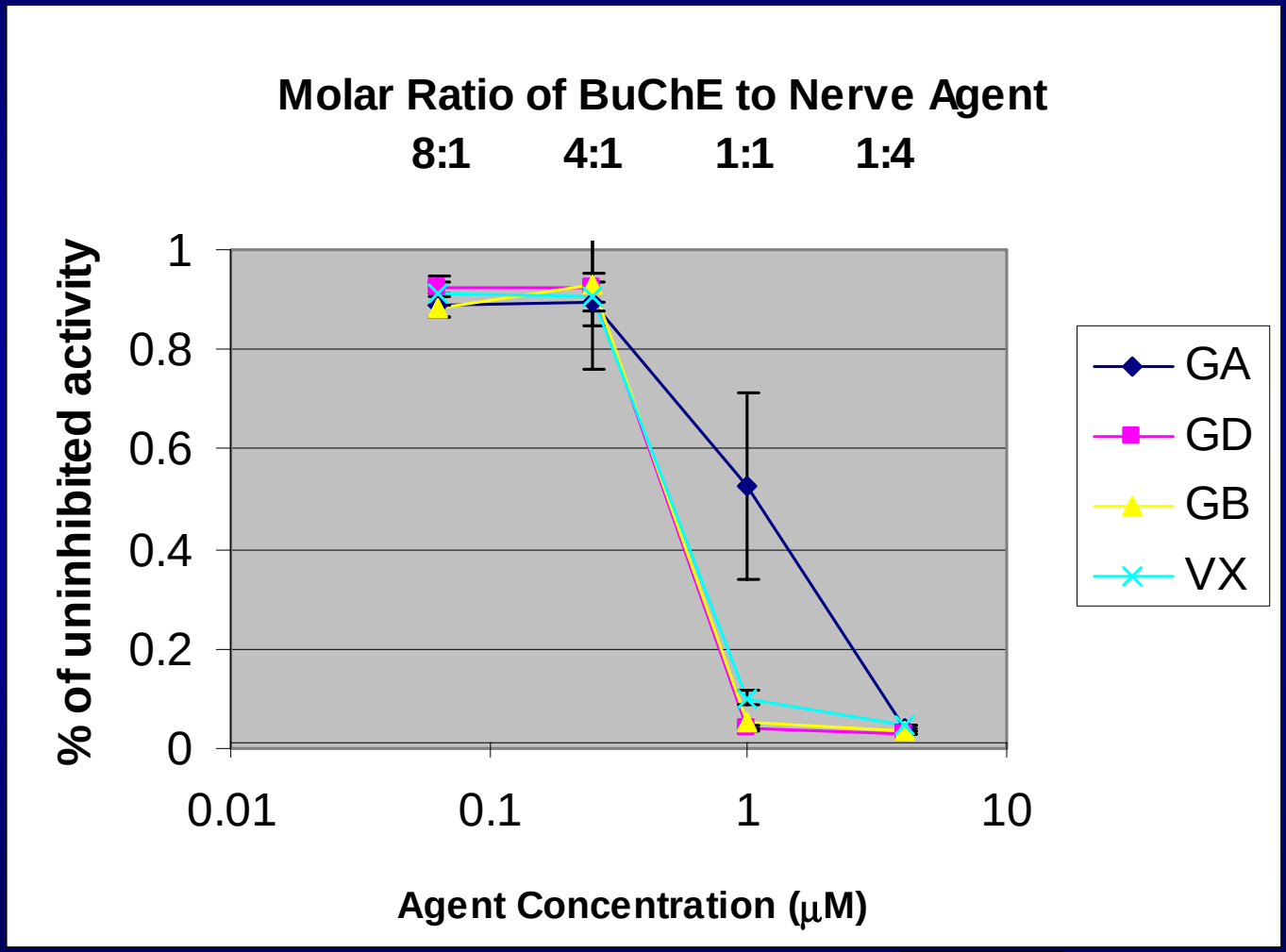
Protexia™ Binds H^3-DFP



Protexia™ BuChE is Inhibited by OPs



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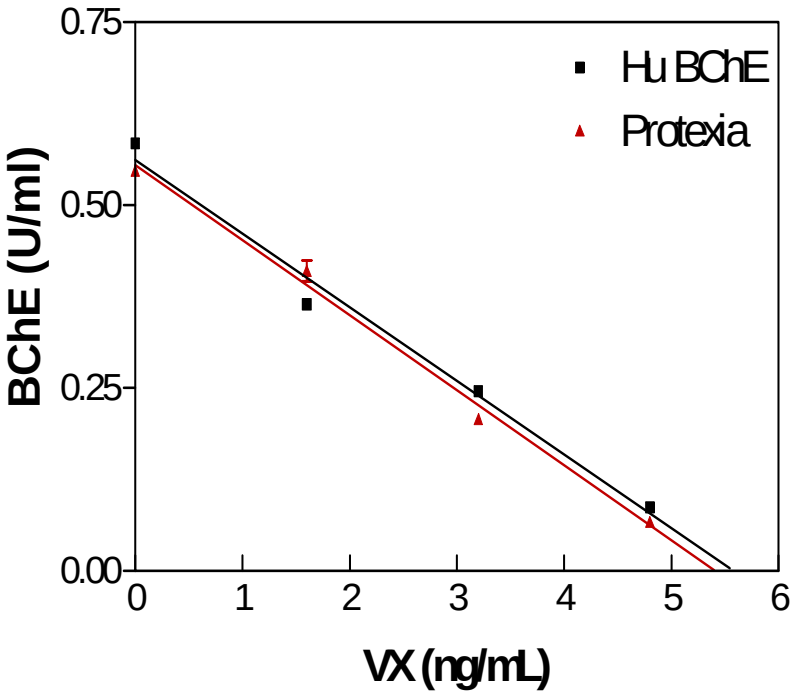
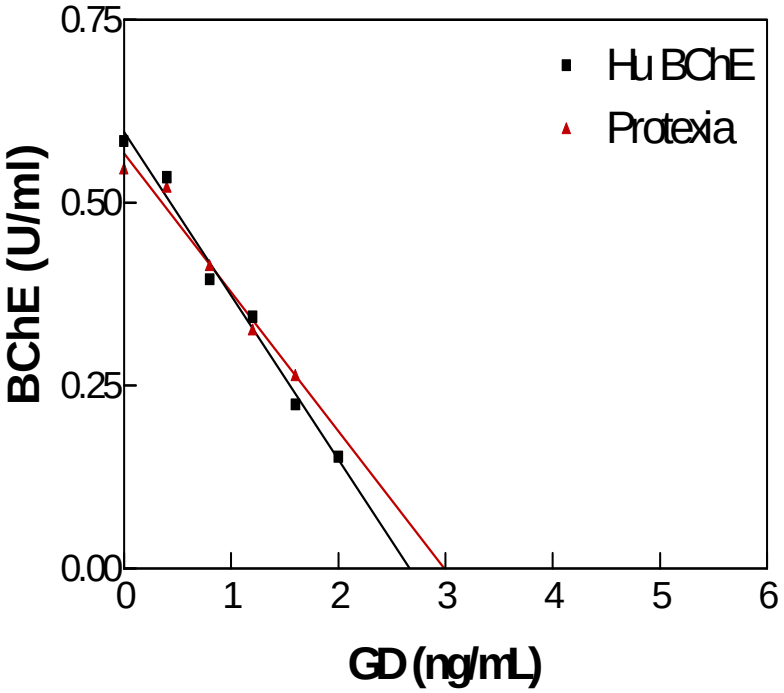
Titration of h-BuChE and Protexia™ by Soman and VX





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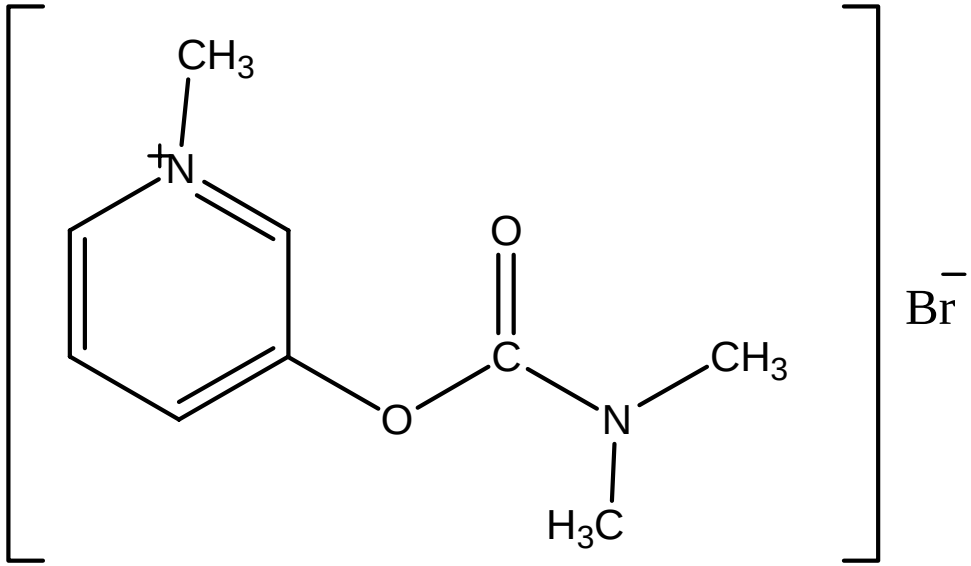
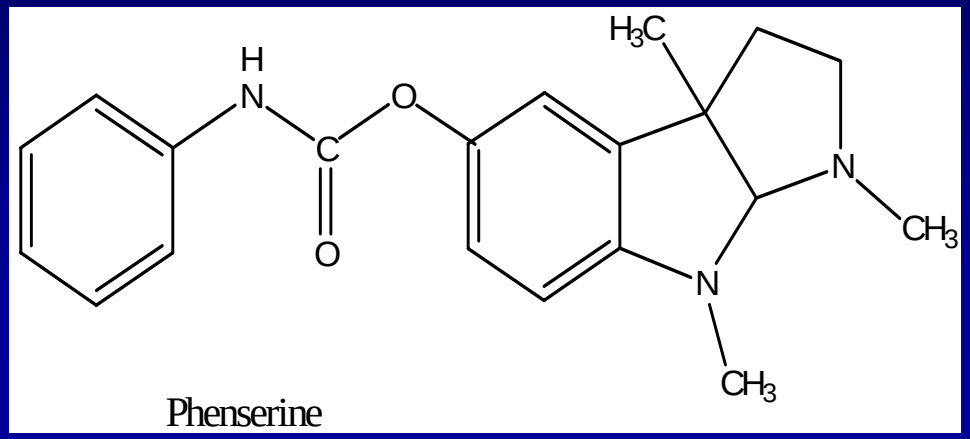
Structures of Inhibitors

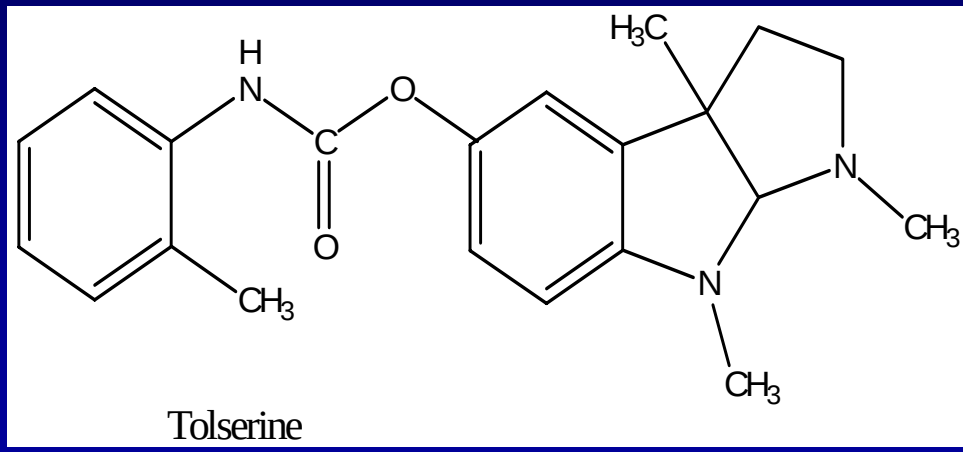
Pyridostigmine bromide



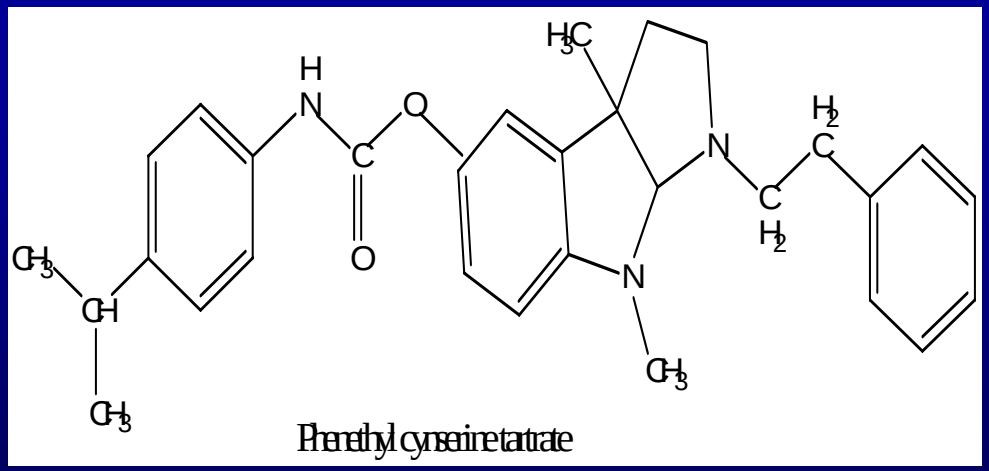
Structures of Inhibitors



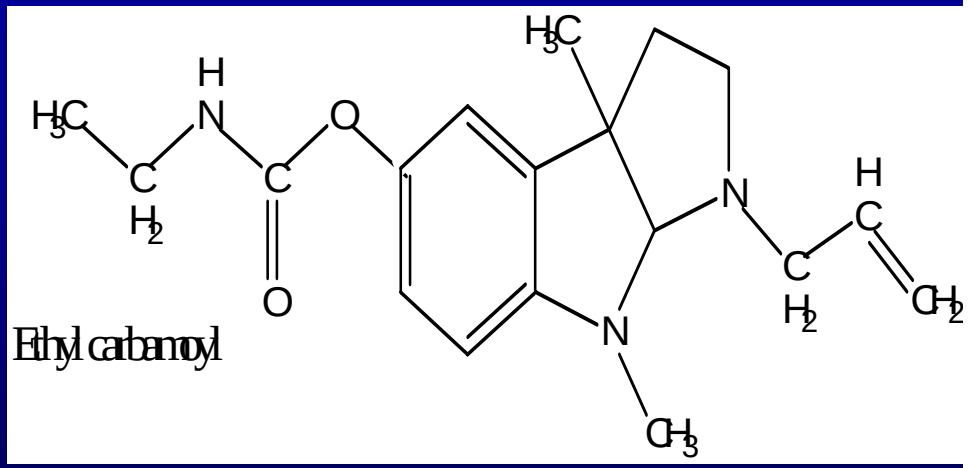
Phenserine



Tolserine



Phenethylcymserine tartrate



Ethylcarbamoyl

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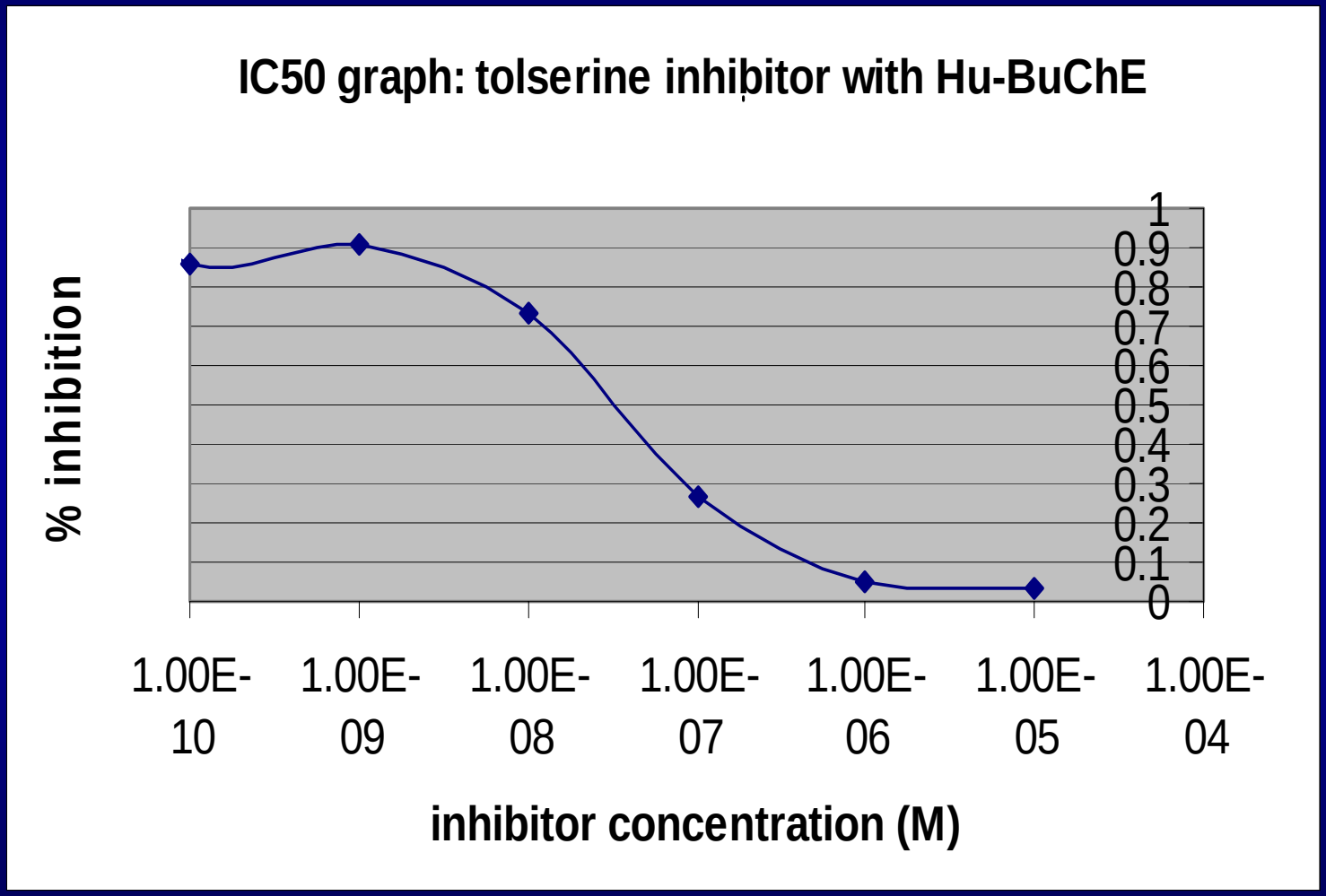
Tolserine with h-BuChE



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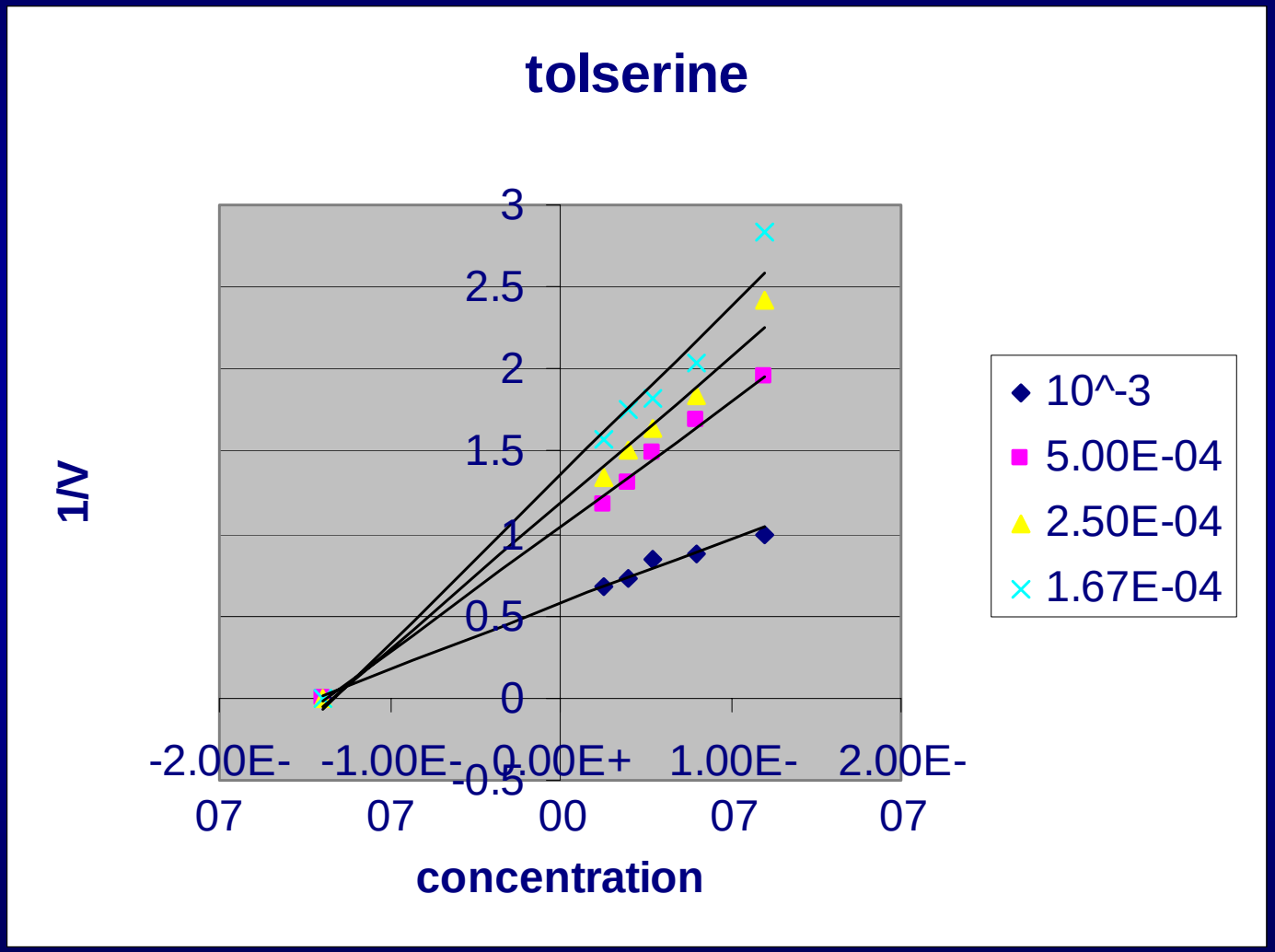
Tolserine with h-BuChE



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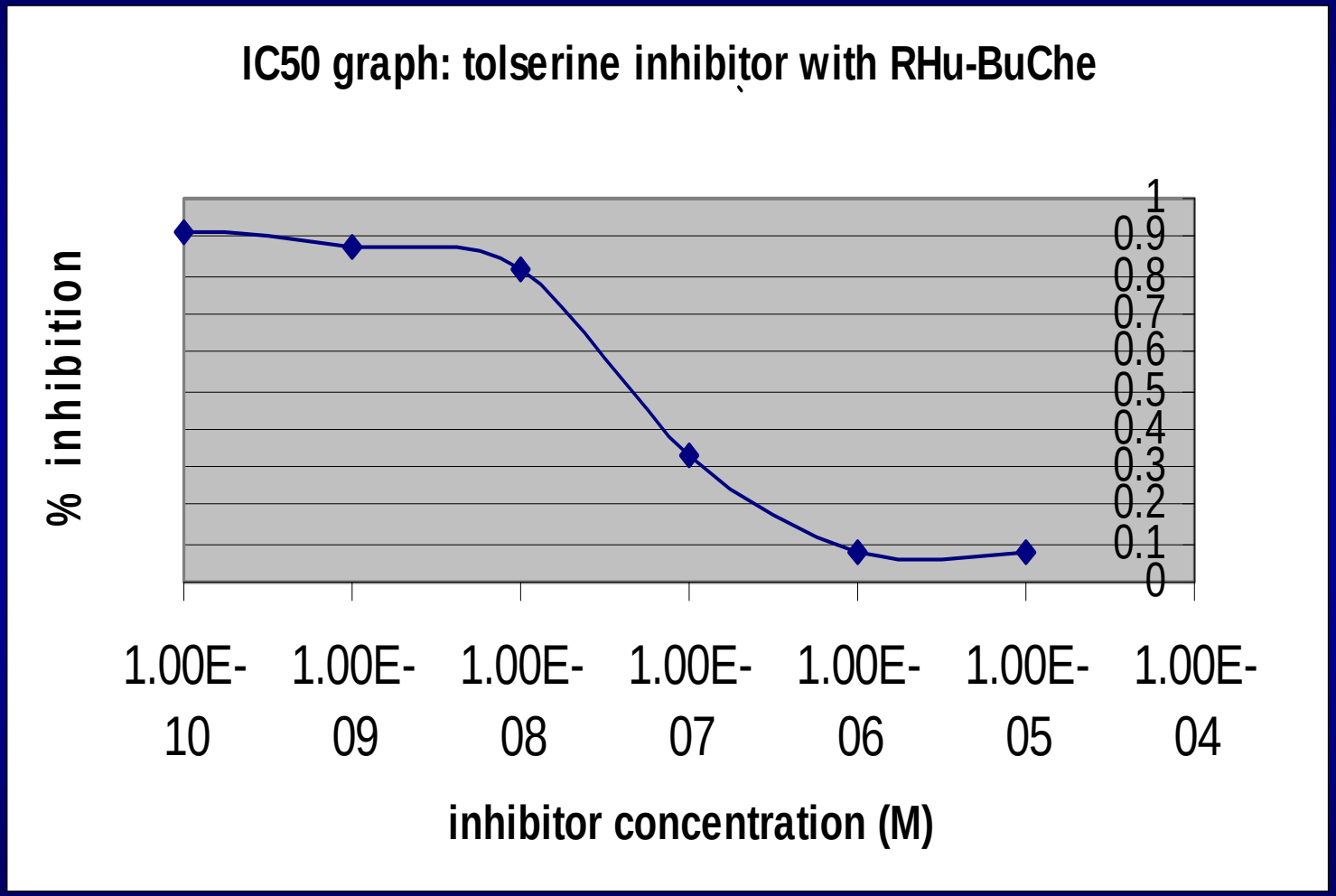
Tolserine with RHu-BuChE (Protexia™)



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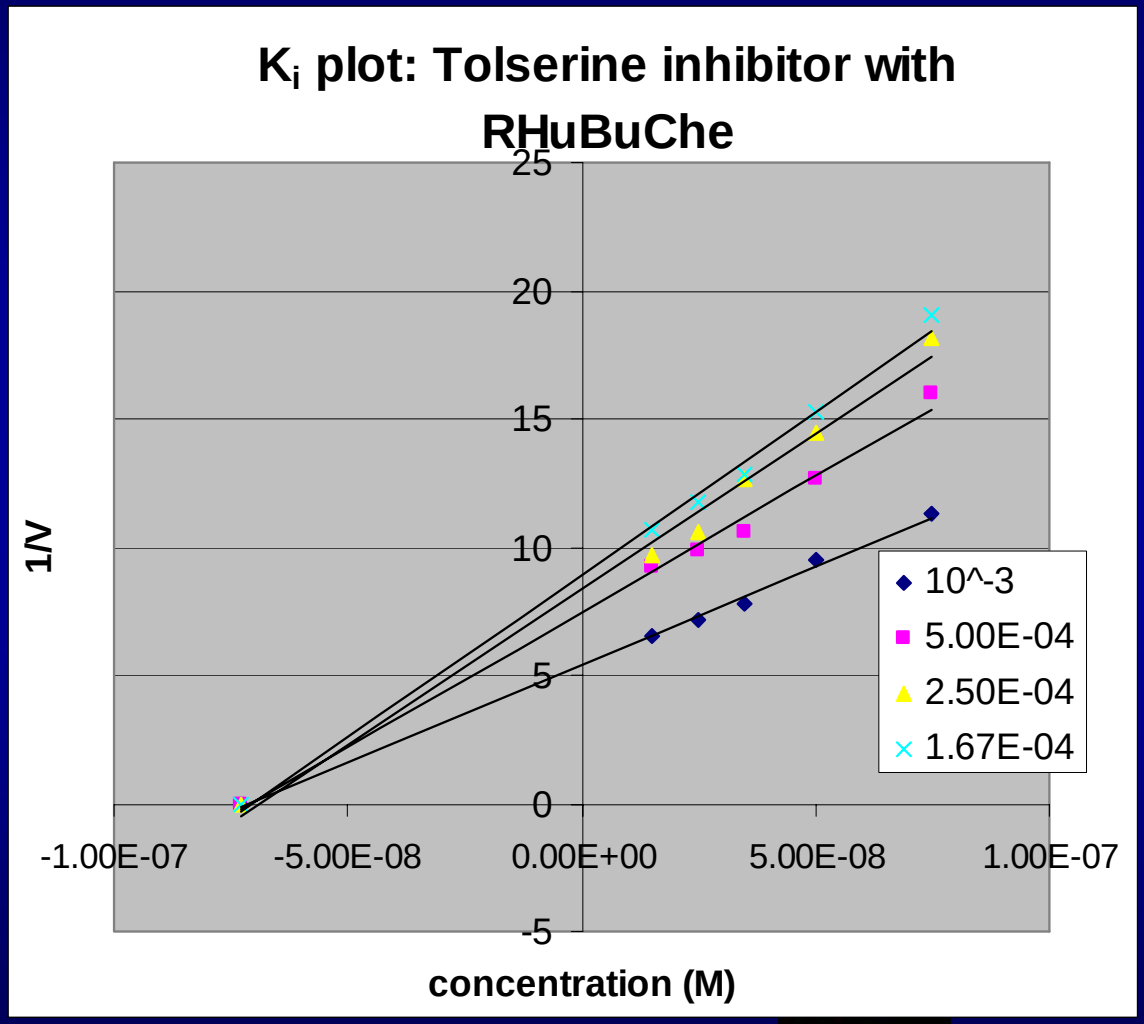
Tolserine with Protexia™



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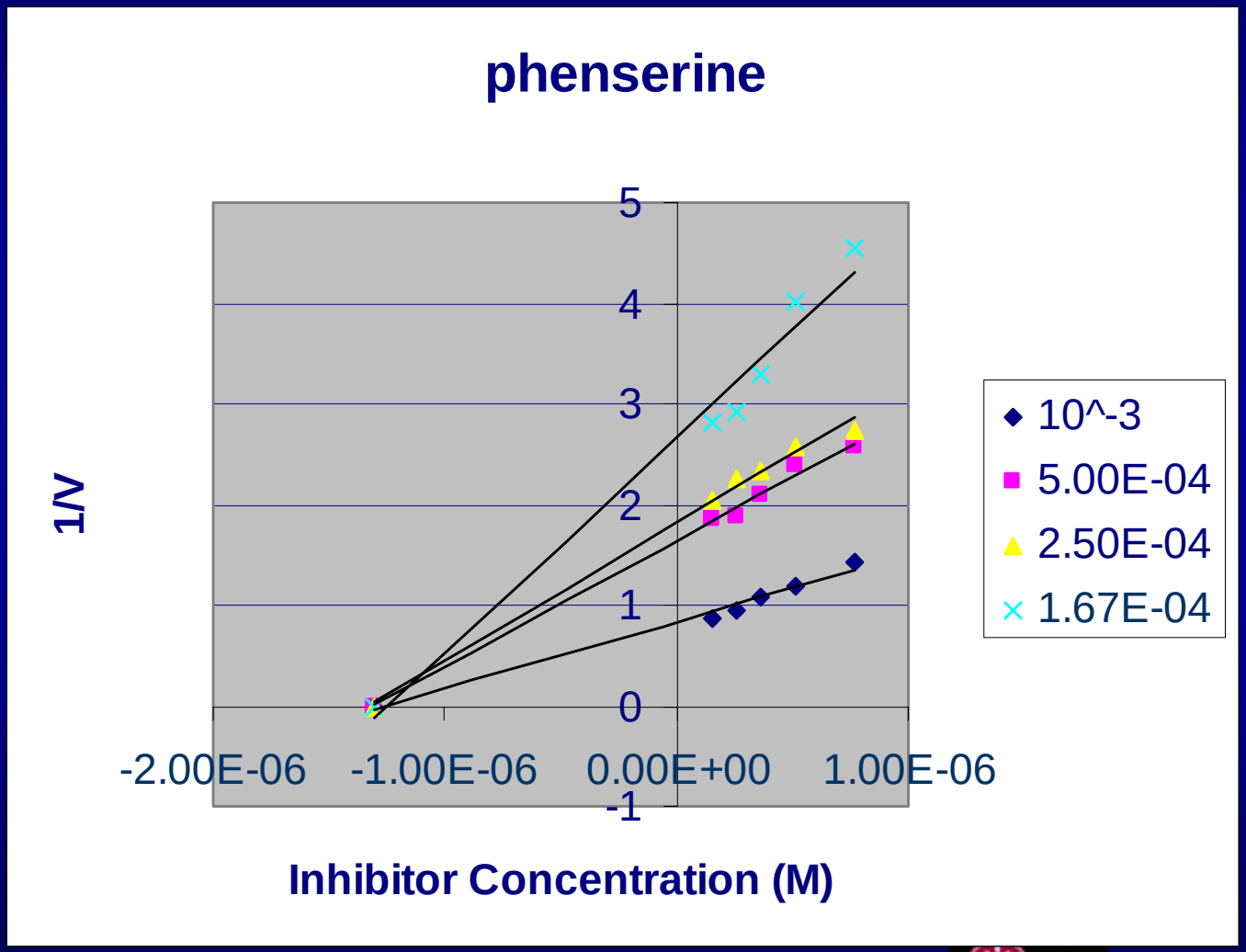
Phenserine with h-BuChE



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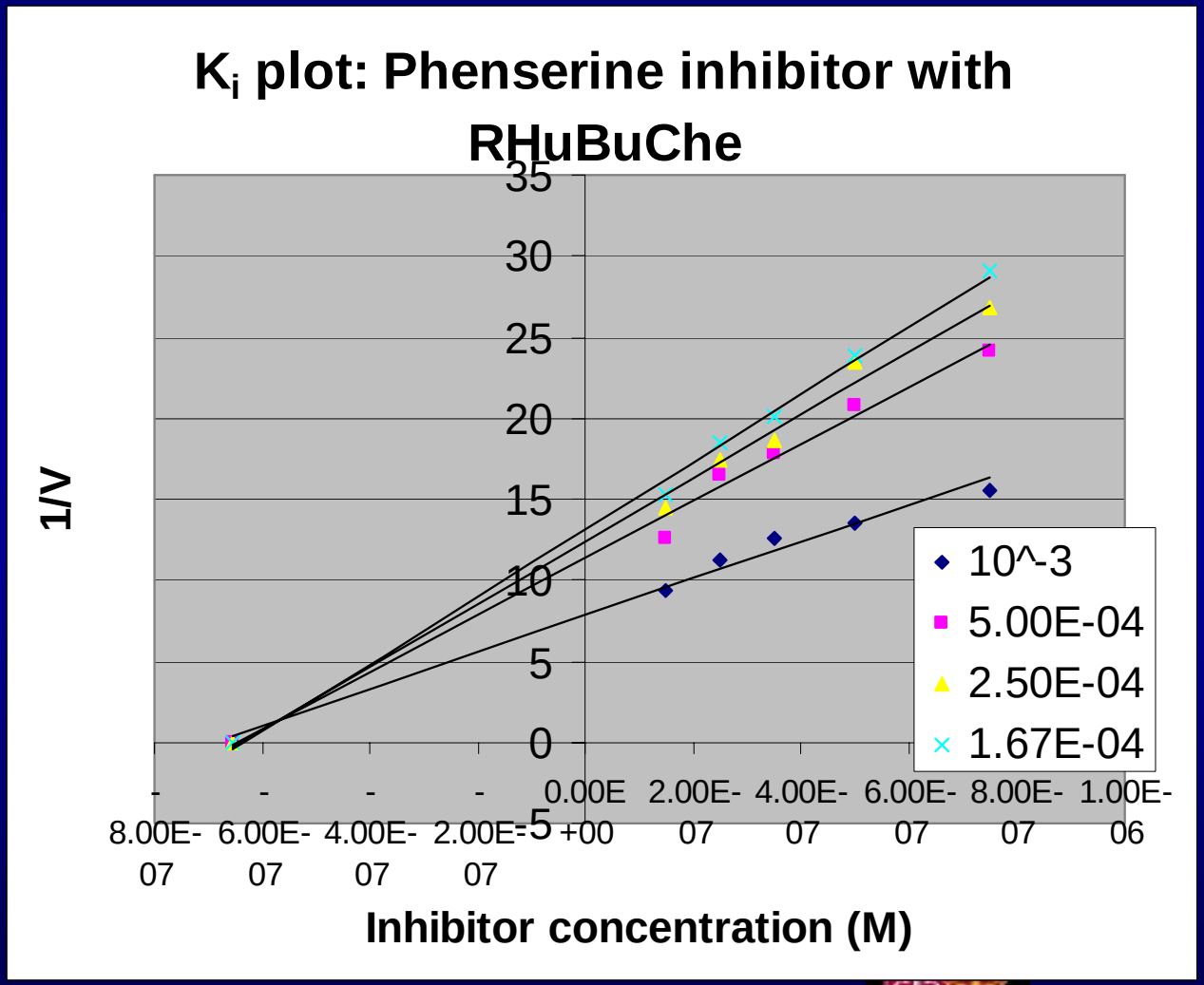
Phenserine with Protexia™



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K_i and IC50 data for Set of Inhibitors

Inhibitor compound	Protexia™ IC 50	Protexia™ K_i (M)	Hu-BuChE IC 50	Hu-BuChE K_i (M)
Phenserine	5×10^{-7}	1.3×10^{-6}	5×10^{-7}	6.6×10^{-7}
Tolserine	8×10^{-8}	1.4×10^{-7}	5×10^{-8}	7.3×10^{-8}
Phenethyl cynserine	5×10^{-11}	5.1×10^{-10}	1×10^{-10}	1.8×10^{-8}
Ethyl carbamoyl	5×10^{-11}	6.2×10^{-10}	5×10^{-14}	1.9×10^{-13}
Pyridostigmine bromide	5×10^{-7}	4.6×10^{-7}	3×10^{-7}	6.2×10^{-7}

Comparison of Hu-BuChE and ProtexiaTM

- In vitro comparison of both forms of human BuChE with a variety of inhibitors

- Properties of recombinant HuBuChE from milk of transgenic goats (Protexia™)

  - **Binds the nerve agents GA, GB, GD, VX**
  - **In vitro properties similar to human plasma BuChE**

- Results to date support similarity in the two sources.

Summary

Plasma BuChE is current standard.

- **Provides protection**
- **PK known in three species**

Protexia™ binds all nerve agents

- **Binding is active site specific**
- **Reaction with a variety of inhibitors is the same for both types of BuChE**

While further characterization of Protexia™ is needed to include PK and efficacy data, in vitro tests suggest both forms of the enzyme behave in a similar manner.